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DUPONT HASKELL GLOBAL CENTERS FOR HEALTH & ENVIRONMENTAL SCIENCES
Discovery Toxicology Group

Biopersistence and Pharmacokinetic Screen in the Rat

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DUPONT REPORT NUMBER:	24281
TESTING SOP NUMBER:	BT004-T-002
STUDY START DATE:	13-June-2007
STUDY END DATE:	21-June-2007
NOTEBOOK(s):	E-111389-AV
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STUDY DESIGN

Test Substance:	HFPO Dimer Acid Ammonium Salt
Lot/Batch Number:	111593-74
Purity:	84.5% (dose corrected for purity)
Species:	Rat
Strain:	Crl:CD(SD)
Vendor:	Charles River Laboratories, Raleigh, North Carolina, U.S.A.
Sex:	Male and Female
Route:	Oral
Age at Study Start:	7-12 weeks
Total Group Size:	3/sex/dose level
Dose Frequency:	Single dose
Dose Vehicle:	Water
Dose:	Low dose 10 mg/kg, high dose 30 mg/kg
Dose Volume:	4 mL/kg
Blood Sample Time Points:	0, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours

OBJECTIVE

To generate preliminary pharmacokinetic data. Fat and liver were analyzed for parent compound to provide an estimate of tissue:plasma ratio.

METHODS

LC/MS Sample Analyses

A. Plasma Samples

The plasma samples were received and stored frozen prior to laboratory use. The samples were prepared for analysis by pipeting 150 µL acetonitrile into a 1.7 mL microcentrifuge tube, and pipeting 50.0 µL of plasma sample. The sample tubes were then vortexed for 1 minute and centrifuged at 14,000 RCF for 30 minutes at 20 °C temperature. After centrifugation, 100 µL of sample supernatant was placed into a HPLC vial and 400 µL of HPLC grade water was added and mixed. As necessary, additional sample dilutions were performed using the 15% acetonitrile in HPLC grade water solvent to ensure that the sample responses were within the calibration curve.

B. Liver and Fat Samples

The liver and fat samples were received and stored frozen prior to laboratory use. The liver tissue samples were extracted in Acetonitrile with 4% perchloric acid while the fat samples were extracted in isopropyl alcohol. The calibration standards were prepared in the appropriate matched solvents.

1. Fat and Liver Extract Preparation.

The fat and liver samples were preprocessed by chopping the tissue samples into small pieces and weighing (0.5 grams) into disposable 15-mL polypropylene centrifuge tubes. Five 5/32" ball bearings were added to each tube, and a pipet was used to add 5000 µL of the appropriate extraction solvent. The tubes were sealed with parafilm, and inserted into a SPEX Certiprep Genogrinder and homogenized for 4 minutes at 1400 strokes/minute. After homogenization, the tubes were centrifuge at 4125 rpm for 20 min at room temperature. The extract supernatant was transferred into glass vials and frozen prior to further sample preparation.

2. Fat and Liver Extract Analysis.

Approximately 100 mg of Envi-Carb graphitized carbon sorbent was placed into a 1.7-mL microcentrifuge tube. A pipet was used to add 50.0 µL of glacial acetic acid directly to the sorbent. Next, a pipet was used to add 1000 µL of sample extract into the centrifuge tube. The tubes were capped, vortexed briefly, and then centrifuged at 10,000 RCF for 10 minutes at room temperature. After centrifugation, a pipet was used to add 525 µL of the 1.7-mL microcentrifuge tube supernatant into a new 1.7 mL microcentrifuge tube and 475 µL of HPLC grade water and mixed. The samples were centrifuged at 10,000 RCF for 5 minutes at room temperature, and supernatant transferred into HPLC vials for analysis. As necessary, additional sample dilutions were performed using the matched solvent to ensure that the sample responses were within the calibration curve.

The prepared samples were analyzed by LC/MS using the following parameters:

HPLC Instrument:	Agilent Model 1100
MS Instrument:	Quattro Micro, Micromass

LC Parameters:

Column:	Zorbax RX-C8, 150 x 2.1 mm, 5 µm particle size
Mobile Phase:	A: 0.15% Acetic acid in NANOpure® water B: 0.15% Acetic acid in Acetonitrile
Column Temperature:	30°C
Injection Volume:	50 µL

MS Parameters:

Capillary Voltage:	3.20 kV
Source Temperature:	120°C
Desolvation Temperature:	300°C
Cone Gas Flow:	40 L/Hr
Desolvation Gas Flow:	400 L/Hr
Collision Gas and Pressure:	Argon, 0.00391 mbar
Ionization Mode:	Electrospray, Negative Ion
Divert Valve:	0-3 minutes to waste, 3-end to source
Data Acquisition Function:	MRM of mass pair: 329.0 → 285.0 0.0 to 6.0 minutes plasma 0.0 to 11 minutes liver, fat
Collision Energy:	5 eV
Dwell	0.100 seconds
Cone Voltage:	9 V

HPLC Gradient (Plasma Samples)

Total Time (min)	Flow Rate (mL/min)	A(%)	B(%)
0.00	0.600	90	10
0.50	0.600	90	10
2.50	0.600	5	95
4.00	0.600	5	95
4.10	0.600	90	10
6.00	0.600	90	10

HPLC Gradient

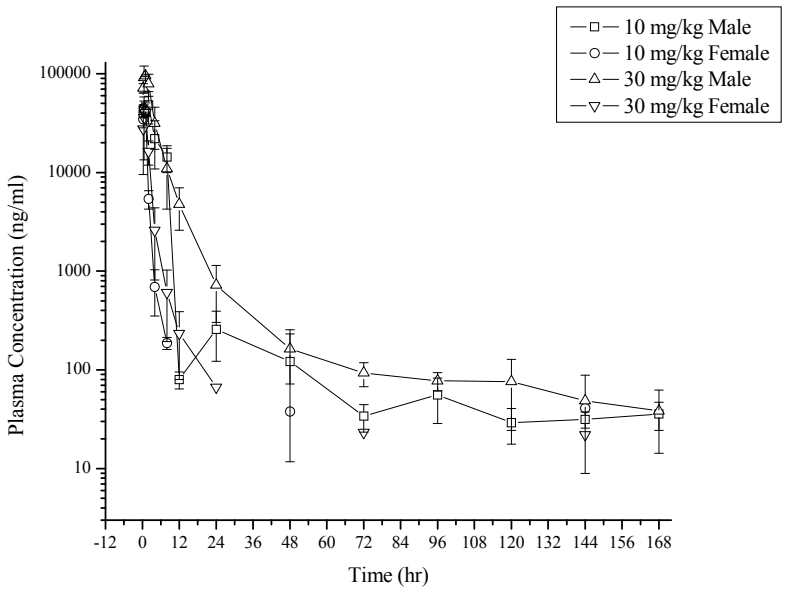
Total Time (min)	Flow Rate (mL/min)	A(%)	B(%)
0.00	0.600	90	10
1.50	0.600	90	10
6.00	0.600	5	95
8.00	0.600	5	95
8.10	0.600	90	10
11.00	0.600	90	10

RESULTS

The individual plasma sample results are provided in Appendix A
The individual liver sample results are provided in Appendix B
The individual fat sample results are provided in Appendix C

Plasma

Graph:



Clearance Time (hr)	10 mg/kg	30 mg/kg
Male	12	22
Female	4	8

Comments: The plasma LOQ was approximately 20 ng/mL. Based on the experimental design, the pharmacokinetic graphs resulting from this class of chemicals makes traditional methods of half-life calculation inappropriate. In order to provide a basis for comparing these chemicals to each other, the clearance time of the analyte will be calculated instead. In traditional pharmacokinetics an analyte is considered to be completely cleared after 98.4% of the analyte is cleared from the plasma.

Tissue/plasma ratio at sacrifice

Fat: All fat samples below LOQ for males and females.

Liver: 10 mg/kg male = 2.2; 30 mg/kg males = 0.8. Female plasma sample were below LOQ at sacrifice so T:P ratio not calculated.

Comments: The tissue LOQ was approximately 20 ng/g.

Appendix A
Individual LC/MS Plasma Sample Results

10 mg/kg results

H-28072 Plasma Concentration for the specified timepoint (ng/mL)

Rat Number	Predose	15 min	30 min	1 Hour	2 Hour	4 Hour	8 Hour	12 Hour	24 Hour	48 Hour	72 Hour	96 Hour	120 Hour	144 Hour	168 Hour
Rat #1	<LOQ	36000	42400	39400	60800	33500	18200	97.5	2310	248	37.3	70.3	42.4	37.1	35.3
Rat #2	<LOQ	43900	53700	45000	56100	21800	15200	73.9	354	71.5	42.6	24.4	24.7	25.4	47.3
Rat #3	<LOQ	47200	37000	50900	28400	11000	9620	68.0	162	45.9	22.1	72.5	20.6	32.3	24.6
Rat #4	<LOQ	39300	40900	43500	4260	776	176	1380	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	41.0	<LOQ
Rat #5	<LOQ	37000	49200	45000	6550	983	168	2810	<LOQ	37.9	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Rat #6	<LOQ	27500	40800	37300	5410	318	217	11300	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

30 mg/kg results

H-28072 Plasma Concentration for the specified timepoint (ng/mL)

Rat Number	Predose	15 min	30 min	1 Hour	2 Hour	4 Hour	8 Hour	12 Hour	24 Hour	48 Hour	72 Hour	96 Hour	120 Hour	144 Hour	168 Hour
Rat #1	<LOQ	78100	121000	93500	88000	38400	10800	5720	765	264	No sample	106	87.4	135	94.2
Rat #2	<LOQ	63100	65400	96500	93100	41300	17700	6340	1120	140	109	86.5	53.9	28.9	55.3
Rat #3	<LOQ	73600	88100	96500	56200	15000	4350	2290	283	86.3	63.9	59.0	39.2	22.6	21.3
Rat #4	<LOQ	43400	59700	44500	11200	611	148	118	67.8	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Rat #5	<LOQ	7800	15100	38200	19400	4080	727	175	66.7	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Rat #6	<LOQ	31800	32900	38700	18600	3120	953	409	<LOQ	<LOQ	23.3	<LOQ	<LOQ	22.0	<LOQ

Note: Rats 1-3 are male, 4-6 are female.

Appendix B
Individual LC/MS Liver Sample Results

		Liver Conc (ng/g)	Plasma Conc (ng/mL)	T:P ratio	Average T:P ratio	S.D. T:P ratio
Male	10 mg/kg Rat #1	43.6	35.3	1.24		
	10 mg/kg Rat #2	90.5	47.3	1.91		
	10 mg/kg Rat #3	84.2	24.6	3.42	2.19	1.12
Female	10 mg/kg Rat #4	54.1	<LOQ	NA		
	10 mg/kg Rat #5	<LOQ	<LOQ	NA		
	10 mg/kg Rat #6	20.6	<LOQ	NA	NA	NA
Male	30 mg/kg Rat #1	48.7	94.2	0.52		
	30 mg/kg Rat #2	44.7	55.3	0.81		
	30 mg/kg Rat #3	21.8	21.3	1.02	0.78	0.25
Female	30 mg/kg Rat #4	<LOQ	<LOQ	NA		
	30 mg/kg Rat #5	<LOQ	<LOQ	NA		
	30 mg/kg Rat #6	<LOQ	<LOQ	NA	NA	NA

Appendix C
Individual LC/MS Fat Sample Results

		Fat Conc (ng/g)	Plasma Conc (ng/mL)	T:P ratio	Average T:P ratio	S.D. T:P ratio
Male	10 mg/kg Rat #1	<LOQ	35.3	NA		
	10 mg/kg Rat #2	<LOQ	47.3	NA		
	10 mg/kg Rat #3	<LOQ	24.6	NA	NA	NA
		<LOQ				
Female	10 mg/kg Rat #4	<LOQ	<LOQ	NA		
	10 mg/kg Rat #5	<LOQ	<LOQ	NA		
	10 mg/kg Rat #6	<LOQ	<LOQ	NA	NA	NA
		<LOQ				
Male	30 mg/kg Rat #1	<LOQ	94.2	NA		
	30 mg/kg Rat #2	<LOQ	55.3	NA		
	30 mg/kg Rat #3	<LOQ	21.3	NA	NA	NA
		<LOQ				
Female	30 mg/kg Rat #4	<LOQ	<LOQ	NA		
	30 mg/kg Rat #5	<LOQ	<LOQ	NA		
	30 mg/kg Rat #6	<LOQ	<LOQ	NA	NA	NA